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RESEARCH PAPER

Differences between the abilities of tegaserod and motilin receptor agonists to stimulate gastric motility *in vitro*

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Background and purpose: Motilin or 5-HT_4 receptor agonists stimulate gastrointestinal motility. Differences in activity are suggested but direct comparisons are few. A method was devised to directly compare the gastric prokinetic activities of motilin, the motilin receptor agonist, erythromycin, and the 5-HT_4 receptor agonist, tegaserod.
Experimental approach: Gastric prokinetic-like activity was assessed by measuring the ability to facilitate cholinergically-mediated contractions evoked by electrical field stimulation (EFS) in rabbit isolated stomach. Comparisons were made between potency, maximal activity and duration of responses.
Key results: Rabbit motilin (r.motilin) 0.003–0.3μM, [Nle^{13}]motilin 0.003–0.3μM, erythromycin 0.3–10μM and tegaserod 0.1–10μM caused concentration – dependent potentiation of EFS-evoked contractions. The potency ranking was r.motilin = [Nle^{13}]motilin > tegaserod > erythromycin. The E_{max} ranking was r.motilin = [Nle^{13}]motilin = erythromycin > tegaserod. Responses to r.motilin and [Nle^{13}]motilin faded rapidly ($t_{1/2}$ 9 and 11 min, respectively) whereas those to erythromycin and tegaserod were maintained longer ($t_{1/2}$ 24 and 28 min). The difference did not appear to be due to peptide degradation. A second application of [Nle^{13}]motilin was excitatory after 60 min contact and fade of the initial response (responses to 0.03 and 0.1μM [Nle^{13}]motilin were not different from those caused by the first application).
Conclusions and implications: Prokinetic-like activities of the 5-HT_4 agonist tegaserod and the motilin receptor agonists were compared by measuring changes in cholinergically-mediated contractions. This novel approach highlighted important differences between classes (greater E_{max} of motilin, compared with tegaserod) and for the first time, within each class (short $t_{1/2}$ for motilin, compared with erythromycin).
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Keywords: stomach; rabbit; erythromycin; tegaserod; 5-HT_4; motilin; prokinetic

Abbreviations: EFS, electrical field stimulation; E_{max}, maximally effective response; r.motilin, rabbit motilin

Introduction

Drugs that act as agonists at 5-hydroxytryptamine$_4$ receptor (5-HT_4) (Sanger, 1998; Quigley, 2000; Langlois and Fischmeister, 2003) or motilin (DiBaise and Quigley, 1999; Peeters, 1999; Park *et al*., 2006) receptors increase gastrointestinal (GI) motility and are indicated as treatments for bowel conditions in which there is a need to stimulate propulsive activity. Both classes of drug are now believed to exert this prokinetic activity mostly because of an ability to facilitate enteric cholinergic function by acting at a pre-junctional site to increase acetylcholine (ACh) release. For the 5-HT_4 receptor agonists such as metoclopramide, cisapride or tegaserod, such activity has been demonstrated in different GI preparations isolated from several different species, including guinea-pig, rat, mouse and human (e.g., Sanger 1985a, b, 1987; Craig and Clarke 1990; Tonini *et al*., 1992; Bassil *et al*., 2005). For the motilin receptor, equivalent studies are less numerous, partly because the existence of this receptor is species-dependent, with orthologues identified in humans and rabbits (Feighner *et al*., 1999; Dass *et al*., 2003), but not in rats or mice (Hill *et al*., 2002; Aerssens *et al*., 2004). In addition, motilin receptor agonists can directly increase smooth muscle tension and only recently have studies begun to focus on the more potent abilities of these agents to facilitate cholinergic function, as a more appropriate pathway by which a coordinated increase in gastric emptying can be effected. For example, in human volunteers the gastric prokinetic effect of a low, but not a high dose of

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erythromycin may be reduced by coadministration of atropine (Coulie *et al*., 1998). In rabbit isolated stomach, low concentrations of motilin, erythromycin and related compounds increase cholinergically mediated, electrically evoked contractions, without similarly increasing contractions mediated by carbachol or exogenously applied ACh (Van Assche *et al*., 1997; Dass *et al*., 2003; Depoortere *et al*., 2003), suggesting an enteric, pre-junctional site of activity. In these studies, only relatively high concentrations of the motilin receptor agonists directly contracted the muscle.

The abilities of both the 5-HT_4 and motilin receptor agonists to increase enteric cholinergic activity in isolated gastric tissues suggests that it is possible to compare these activities and make predictions on the relative gastric prokinetic activities of the different classes of drug. A difference in therapeutic efficacy between motilin and 5-HT_4 receptor agonists has previously been suggested by clinical studies in which a more effective increase in gastric emptying rate was shown in response to erythromycin, when compared with metoclopramide, cisapride or the peripherally restricted dopamine D_2 receptor antagonist domperidone (Annese *et al*., 1997; Sturm *et al*., 1999). Others have described smaller (Zatman *et al*., 2001) or no advantages (Erbas *et al*., 1993) of erythromycin over metoclopramide, although the effects of different doses of these drugs were not explored. Boivin *et al*. (2003) described similar abilities of 3 mg/kg intravenous erythromycin and 10 mg metoclopramide to increase gastric emptying in volunteers, but the adverse events associated with these doses (nausea/stomach cramping during erythromycin infusion, drowsiness with metoclopramide) makes it difficult to draw valid conclusions about the relative therapeutic efficacies of these two pharmacological approaches. In dogs, similar abilities of erythromycin and cisapride to increase gastric emptying have been reported (Cowles *et al*., 2000).

In our experiments, we began by demonstrating that the rabbit isolated gastric antrum, a preparation sensitive to the ability of motilin to increase cholinergic activity, can also detect a similar activity induced by 5-HT_4 receptor agonists (Corcoran *et al*., 2004). We have now refined the assay technique to optimize detection of large ranges in excitatory activity, and then for the first time, directly compared the abilities of motilin, erythromycin and tegaserod to increase cholinergically mediated contractions of the stomach, looking for differences in maximal activity and duration of response; a need for the latter measurement is indicated by the suggested clinical tolerance to repeat doses of high, but not necessarily, to low doses of erythromycin (Dhir and Richter, 2004). By focussing on the abilities of low concentrations of these receptor agonists to potentiate cholinergically mediated contractions, rather than using higher concentrations of motilin or erythromycin to evoke muscle contraction directly (e.g., see Dass *et al*., 2003; Thielemans *et al*., 2005), our findings suggest that important differences exist both within each class (different response kinetics for motilin, compared with erythromycin) and between classes (greater E_{max} of motilin receptor agonists, compared with tegaserod). These novel findings improve our understanding of how peptide and non-peptide ligands may interact with the motilin receptor and, in addition, help to clarify the therapeutic potential of these different classes of prokinetic drugs.

Methods

Test systems used

The methods were modified from those described previously in detail (Dass *et al*., 2003). In brief, adult male New Zealand white rabbits (1.5–2.5 kg) were killed by pentobarbitone overdose followed by cervical dislocation. All efforts were made to minimize the number of animals used and animals were killed in accordance with the UK Animals (Scientific Procedures) Act 1986. Following a midline incision, whole stomachs were blunt-dissected and placed immediately in ice-cold Krebs solution (NaCl 121.5, $CaCl_2$ 2.5, KH_2PO_4 1.2, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 5.6 mM) previously equilibrated with 5% CO_2/95% O_2. The gastric antrum was removed, cut open and the mucosa removed by dissection. Strips of tissue were cut ($\sim 10 \times \sim 2$ mm) parallel to the circular muscle and suspended between two parallel platinum ring electrodes in 5 ml tissue baths containing Krebs solution bubbled with 5% CO_2/95% O_2, maintained at pH 7.4 ± 0.1 and 37°C.

Measurements made

Tension was measured using the Dynamometer UF1 force–displacement transducers (Pioden Control Ltd, UK). Data acquisition and analysis were performed using MP100 hardware and AcqKnowledge software (Biopac Systems, Inc., USA). Tissues were initially suspended under 3 *g* tension and allowed to equilibrate for at least 45 min during which time bath solutions were changed every 15 min. During this time, muscle tension stabilized at ~ 1 *g*. Electrical field stimulation (EFS) was carried out by using biphasic square-wave pulses of 0.5 ms width, 10 Hz frequency, 50 V for 30 s every 1 min using multichannel stimulators (Scientifica Ltd, UK). EFS was applied using these parameters, over a 30-min period, after which the bath solution was changed. The frequency was then adjusted every 10 min between 1 and 14 Hz, so that the tissues generated an increase in muscle tension greater than 0.1 *g* but less than 1 *g*. Thereafter the frequency of stimulation remained fixed.

Experimental design

After obtaining consistent EFS-evoked contractions for 10 min, a single concentration of drug or vehicle was added to each strip and left in contact over 60 min, in order to observe both the magnitude and the duration of the evoked response. For data analysis, following compound administration the averages of three consecutive responses to EFS were measured over a 60 min period to give 20 separate data points. Changes in the responses to EFS were then expressed as a percentage of the mean of at least five pre-dose responses (basal activity). In additional experiments with [Nle^{13}]motilin, the same concentration of the peptide was re-applied

at the end of the 60 min exposure period, to determine if further response could be generated. Responses to [Nle^{13}]motilin were also examined in the presence of the peptidase inhibitors, amastatin (10 μM), phosphoramidon (10 μM) and bestatin (10 μM), added into the bathing solution 30 min before the application of a single concentration of 30 nM [Nle^{13}]motilin and left in contact for 60 min. Finally, additional work was carried out to confirm 5-HT$_4$ receptor activation by tegaserod. In these experiments, the selective and non-surmountable 5-HT$_4$ receptor antagonist SB-204070A 1 μM (Wardle *et al*., 1994) was added to each preparation, 10 min after obtaining consistent EFS-evoked contractions. The antagonist was left in contact for 15 min before the application of a single concentration of 3 μM tegaserod, left in contact for 60 min.

To examine for any post-junctional activity, tegaserod and the motilin receptor agonists were each tested for any ability to affect contractions evoked by carbachol 1 μM, a submaximally effective concentration, which evoked contractions approximately similar to the amplitude of the contractions evoked by EFS (data not shown). Carbachol was applied to the tissues every 20 min (120 s before washout), two to three times until consistent responses were observed. Ten minutes before the next application of carbachol, a maximally effective concentration of [Nle^{13}]motilin (0.3 μM), erythromycin (10 μM) or tegaserod (3 μM) were added to the bathing solution. Changes in the responses to carbachol were calculated as a percentage of the pre-dose response that was expressed as 100%.

Data analysis and statistical procedures

Agonist pEC$_{50}$ values (the negative logarithm to base 10 of the EC$_{50}$ value, which is the concentration of the agonist that produces 50% of the maximal response) were determined by non linear regression using GraphPad Prism software. E_{max} denotes the maximal response achieved by the drug and the time for the response to decline by 50% is equal to the $t_{1/2}$, ($t_{1/2}$; taken from when drug added) Data are expressed as means $\pm$ s.e.m.; n-values are numbers of animals used. Differences between the means were determined using a Student's t-test for unpaired data; $P<0.05$ is considered as statistically significant.

Materials

All drugs were freshly prepared before use. The norleucine^{13} analogue of porcine/human motilin, [Nle^{13}]motilin (Calbiochem, San Diego, CA, USA), was dissolved in distilled water. Erythromycin (Sigma, Gillingham, Dorset, UK) was dissolved in ethanol with subsequent dilutions in distilled water for tissue studies. Carbachol, atropine (Sigma, UK) and the nerve toxin tetrodotoxin (Tocris, Bristol, UK), were also dissolved in distilled water . Rabbit motilin (r.motilin), tegaserod and SB-204070A were made in our laboratories. r.motilin and SB-204070A were dissolved in distilled water and tegaserod was dissolved at 10 mM in 100% dimethylsulphoxide, subsequent dilutions were made in distilled water. The peptidase inhibitors pepstatin, amastatin and bestatin (all from Sigma, UK) were dissolved in distilled water.

Results

In the majority of tissue preparations EFS evoked a monophasic contraction (Figure 1), prevented by adding 1 μM tetrodotoxin ($n=4$, 30 min contact) or 1 μM atropine ($n=4$, 30 min contact; data not shown).

Effects on neuronally mediated contractions

r.motilin (0.003–0.3 μM) concentration-dependently increased the EFS-evoked contractions, the responses fading rapidly back to a time-matched control baseline during the contact period (Figures 1 and 2, Table 1). The maximum potentiation observed was 506$\pm$112% (Figure 3, Table 2). [Nle^{13}]motilin, 0.003–0.3 μM, also increased the amplitude of EFS-evoked contractions, the responses again fading rapidly back to a time-matched control baseline during the contact period (Figures 1 and 2; Table 1). The maximum potentiation observed was 740$\pm$151% (Figure 3, Table 1) and this increase was not statistically different from that evoked by r.motilin. At high concentrations, both r.motilin (10–100 nM) and [Nle^{13}]motilin (30–300 nM) caused a short-lived increase in muscle tension (Figure 1).

The response to [Nle^{13}]motilin was not changed by the combination of the protease inhibitors amastatin, phosphoramidon and bestatin (10 μM each). Thus, in these experiments, the maximum potentiation observed was 662$\pm$232% ($P>0.05$ compared with the maximum potentiation evoked

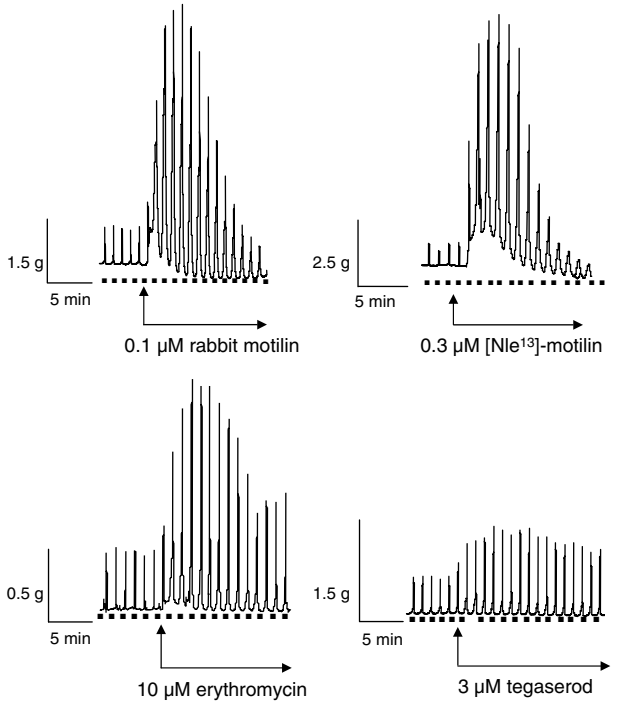

Figure 1 Representative traces showing the effects of r.motilin, [Nle^{13}]motilin, erythromycin and tegaserod on EFS-evoked, nerve-mediated contractions in rabbit isolated gastric antrum circular muscle preparations. EFS (0.5 ms width, 50 V for 30 s, every 1 min) was applied using a fixed frequency (1–14 Hz) that varied between tissues so that the muscle tension generated was greater than 0.1 g but less than 1 g.

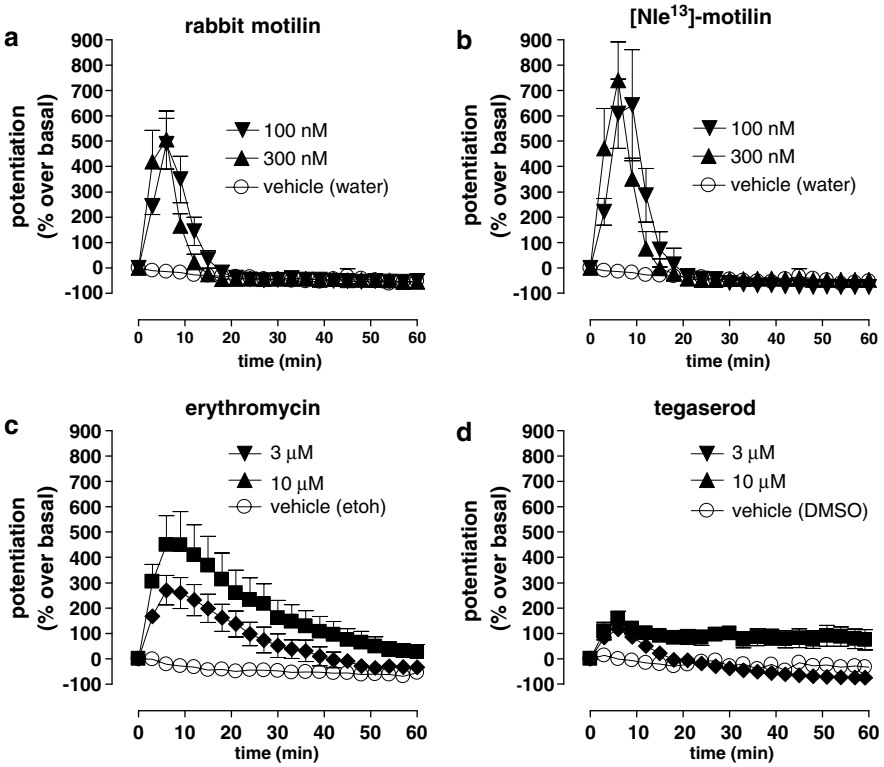

Figure 2 The abilities of r.motilin, [Nle^{13}]motilin, erythromycin and tegaserod to facilitate cholinergically-mediated contractions in rabbit isolated gastric antrum circular muscle preparations, measured over a 60 min period. Following addition of the compound the averages of three consecutive responses to EFS were measured over the 60 min period to give 20 separate data points. Changes in the responses to EFS were then expressed as a percentage of the mean of at least five pre-dose responses (basal activity). Data are expressed as means$\pm$s.e.m.; $n=4$–6. Responses to vehicles and to the maximal and a submaximally-effective concentration are shown for (**a**) r.motilin 100 and 300 nM; (**b**) [Nle^{13}]motilin 100 and 300 nM; (**c**) erythromycin 3 μM and 10 μM and (**d**) tegaserod 3 and 10 μM.

Table 1 The time taken for responses to tegaserod and to motilin receptor agonists to fade in rabbit isolated gastric antrum circular preparations, measuring their ability to facilitate cholinergically mediated contractions

Compound	*Concentration* (n) (μM)	$t_{1/2}$ *values (min)*
r.motilin	0.003 (4)	9.5$\pm$0.9
	0.01 (4)	12.0$\pm$1.2
	0.03 (5)	12.0$\pm$1.3
	0.1(5)	11.4$\pm$0.6
	0.3 (4)	9.0$\pm$0
[Nle^{13}]motilin	0.01 (4)	15.0$\pm$1.2
	0.03 (5)	14.4$\pm$1.1
	0.1 (4)	12.0$\pm$1.2
	0.3 (5)	11.4$\pm$1.5
Erythromycin	1 (4)	34.5$\pm$5.3
	3 (5)	24.0$\pm$5.6
	10 (3)	23.0$\pm$8.7
Tegaserod	0.1 (6)	27.5$\pm$10.4
	0.3 (4)	28.5$\pm$8.9
	1 (6)	42.5$\pm$10.6
	3 (6)	28.0$\pm$12.3
	10 (6)	11.5$\pm$0.9

Abbreviations: r.motilin, rabbit motilin.
After single application of the ligand and during continual incubation, the time taken for the maximum response to fade by 50% ($t_{1/2}$) was measured. (time taken from when the drug was added).

by [Nle^{13}]motilin in the absence of protease inhibitors; $n=3$ and 6, respectively) and the $t_{1/2}$ values (times taken for the maximum response to fade by 50%; see Table 1 for values in the absence of peptidase inhibitors) were unchanged by the presence of the peptidase inhibitors (the $t_{1/2}$ for 0.03 μM [Nle^{13}]motilin in the absence and presence of the peptidase inhibitors was 14.4$\pm$1.1 and 12$\pm$1.7 min, respectively, $P>0.05$, $n=3$ and 6). Finally, in additional experiments with [Nle^{13}]motilin, the same concentration of peptide was re-applied at the end of the 60 min contact period following the first application of [Nle^{13}]motilin. Using this protocol, the amplitude of the EFS-evoked contractions tends to fade with time (Figures 2 and 4), complicating the interpretation of the subsequent response to [Nle^{13}]motilin. Nevertheless, the second application of [Nle^{13}]motilin also increased the amplitude of contractions to EFS (Figure 4). These potentiations were not different from the corresponding values obtained with the same concentrations on first application (at 10 nM, 111$\pm$69%, $n=2$; at 30 nM, 114$\pm$35%, $n=3$; at 100 nM, 187$\pm$82%, $n=2$), However, at the highest concentration of [Nle^{13}]motilin tested (300 nM), re-application induced only 19$\pm$6% ($n=3$) of the increases evoked by the first application of this peptide.

Erythromycin 0.3–10 μM increased EFS-evoked contractions in a manner which at all but the highest concentration, faded partially and at a much slower rate

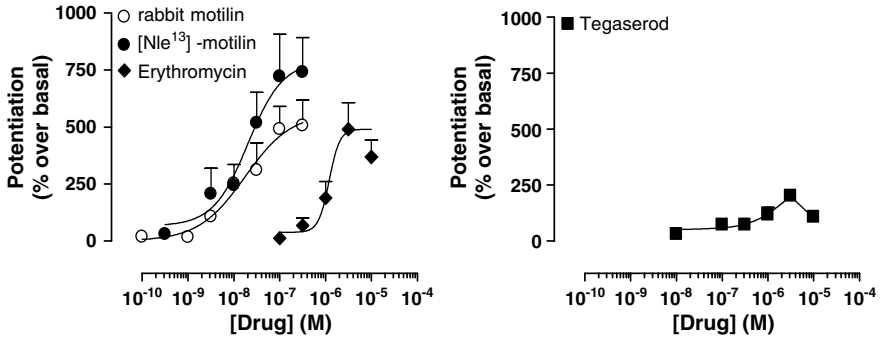

Figure 3 Concentration-dependent facilitation of cholinergically mediated contractions in rabbit isolated gastric antrum circular muscle preparations by r.motilin, [Nle^{13}]motilin, erythromycin and tegaserod. Data shown are the maximum values of potentiation at each concentration and are expressed as means$\pm$s.e.m.; $n=3$–5.

Table 2 Potencies and maximal activities of tegaserod and motilin receptor agonists in rabbit isolated gastric antrum circular preparations

Compound	*Concentration* (n) (μM)	*Apparent* p*EC$_{50}$*	*Maximum response*	
			% increase	*Concentration* (μM)
r.motilin	0.0001–0.3 (4–5)	7.8$\pm$0.4	506$\pm$112	0.3
[Nle^{13}]motilin	0.0003–0.3 (3–6)	7.7$\pm$0.4	740$\pm$151	0.3
Erythromycin	0.1–10.0 (5)	6.0$\pm$0.1	490$\pm$117	3.0
Tegaserod	0.01–10.0 (3–6)	6.5$\pm$0.2	202$\pm$63	3.0

Abbreviations: EFS, electrical field stimulation; r.motilin, rabbit motilin.
Concentration–response curves to these ligands were derived by single applications of different concentrations to separate preparations of stomach, measuring their ability to facilitate cholinergically mediated contractions. Apparent pEC_{50} values are the concentrations which evoke a response which was 50% of the maximum increase in EFS-evoked contractions (maximum response).

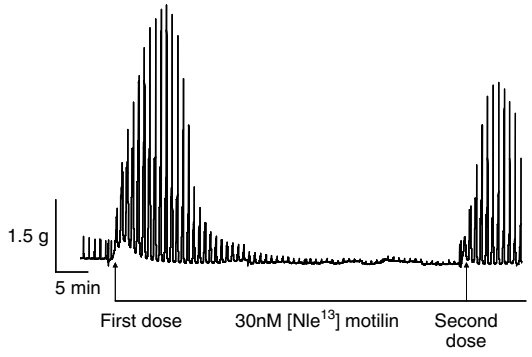

Figure 4 Representative trace showing the ability of a second application of 30 nM [Nle^{13}]motilin to facilitate cholinergically-mediated contractions in rabbit isolated gastric antrum circular muscle preparations. Tissues were initially exposed to 30 nM [Nle^{13}]motilin and left in contact with the peptide for 60 min before re-application of the same concentration of [Nle^{13}]motilin. Using this protocol, the amplitude of the EFS-evoked contractions tends to fade with time.

than that observed using either motilin or [Nle^{13}]motilin, during the 60 min contact period (Figures 1 and 2, Table 1); the maximum potentiation was 490$\pm$117% (Figure 3, Table 2) and this increase was not significantly different from the magnitude of the increase evoked by either r.motilin or [Nle^{13}]motilin. At the highest concentration (10 μM), erythromycin caused a short-lived increase in muscle tension (Figure 1).

Tegaserod (0.1–10 μM) increased EFS-evoked contractions in a manner which did not rapidly fade, although as in the experiments with erythromycin, there was a small loss of response over time and similar apparent $t_{1/2}$ values were determined (Figures 1 and 2; Table 1). The maximum facilitation of EFS-evoked contractions caused by tegaserod was 202$\pm$63% (Figure 3, Table 2) and this was smaller ($P<0.05$ each) than the increases evoked by r.motilin, [Nle^{13}]motilin or erythromycin. In terms of apparent pEC_{50} values, the relative potencies of these ligands was r.motilin = [Nle^{13}]motilin > tegaserod > erythromycin (Table 2). However, these values should be treated with caution, because the excitatory activity of tegaserod and erythromycin exhibited marked bell-shaped concentration–response characteristics (Figure 3). Finally, and in separate experiments, the increase in EFS-evoked contraction caused by a maximally effective concentration of tegaserod (3 μM) was 107$\pm$32 and 15$\pm$7%, respectively, ($P<0.05$, $n=7$) in the absence and presence of the non-surmountable 5-HT$_4$ receptor antagonist SB-204070A (1 μM).

Effects on carbachol-induced contractions

The site of action of [Nle^{13}]motilin, erythromycin and tegaserod was examined by testing the abilities of maximally effective concentrations to modulate submaximal contractions evoked by carbachol 1 μM instead of EFS. Neither [Nle^{13}]motilin (0.3 μM) nor vehicle affected the contractions (306$\pm$110 and 99$\pm$11% of control, respectively; $P>0.05$;

$n=3$–5). This concentration of [Nle^{13}]motilin increased baseline muscle tension. In the presence of erythromycin (10 μM) or tegaserod (3 μM), no consistent changes in baseline muscle tension were observed and the carbachol-induced contractions were 105 ± 23 and $79\pm14\%$ ($P>0.05$; $n=3$–4) and $107\pm19\%$ and $96\pm0.5\%$ ($P>0.05$, $n=2$–3), respectively.

Discussion and conclusions

Our studies have shown that tegaserod and the motilin receptor agonists can increase electrically-evoked, cholinergically-mediated contractions of rabbit isolated gastric antrum. Erythromycin and tegaserod did not increase contractions evoked by carbachol. Although not statistically significant, the application of [Nle^{13}]motilin (0.3 μM) did tend to increase the contractions evoked by carbachol but a simultaneous increase in muscle tension complicates the interpretation of these data. Overall, therefore, these studies suggest that the ability of tegaserod and motilin receptor agonists to increase cholinergically-mediated contractions depends mostly on a pre-junctional site of action within the enteric nervous system. These observations confirm those of others (see Introduction for references), but our study is the first to directly and systematically compare the effects of these two classes of prokinetic agents in the same assay. This assay allowed us to construct concentration–response curves and to measure the maximal activities and durations of the responses evoked. Using these data, we are able to further clarify how each receptor agonist and each class of prokinetic agent may stimulate gastric motility.

In our experimental protocol, we chose parameters of EFS that were the minimum required to evoke a consistent response, relative to background activity. We were, therefore, able to detect and distinguish between different drugs that facilitate cholinergic function by varying degrees. In this design, the variable parameter was frequency and the response to EFS was required to generate an increase in muscle tension greater than 0.1 g but less than 1 g, in order to differentiate evoked-responses from background, spontaneous muscle activity. This requirement, to generate muscle tension within a fixed range minimised variations in the effects of drugs on groups of tissues with large variations in baseline contractile tension and was an improvement on our previous, preliminary study (Corcoran *et al*., 2004). Critically, this experimental protocol made it possible to directly compare, for the first time, the abilities of different classes of gastric prokinetic agents to facilitate gastric cholinergic activity, an activity found to vary widely in terms of both the kinetics of the responses evoked and in the maximal activities observed.

To study the effects of 5-HT$_4$ receptor activation, we used tegaserod, known to act via the 5-HT$_4$ receptor to facilitate cholinergic activity and increase neurotransmitter release and peristalsis in guinea-pig isolated intestine (see Scott and Perry, 1999, for references). However, additional activity as a 5-HT$_{2B}$ receptor antagonist (Beattie *et al*., 2004), coupled with the demonstration of a bell-shaped concentration–response curve in the present experiments, suggested a need to confirm that tegaserod was indeed increasing cholinergic activity in rabbit stomach via an activation of the 5-HT$_4$ receptor. This was achieved by demonstrating antagonism of the excitatory activity of tegaserod by the non-surmountable 5-HT$_4$ receptor antagonist SB-204070A.

To study the effects of motilin receptor activation, we used r.motilin and [Nle^{13}]motilin, which varies in six amino acids from motilin, and erythromycin. [Nle^{13}]motilin is the more stable analogue of porcine motilin (Miyashita *et al*., 1988; Kitazawa *et al*., 1993; Feighner *et al*., 1999) and was used to minimize variations caused by degradation of the peptide (see below for further discussion on the stability of this compound during the present experiments). [Nle^{13}]motilin, porcine and r.motilin have previously been shown to be approximately equipotent excitatory agents in the rabbit gastric antrum, being more potent at exerting prokinetic-like activity than erythromycin, with each behaving as a full or near-full agonist at the receptor (Peeters *et al*., 1986; Van Assche *et al*., 1997; Dass *et al*., 2003). The results of our studies are consistent with these observations. By contrast, others have described an ability of erythromycin to directly inhibit smooth muscle contractility in the gut (Minocha and Galligan, 1991; Depoortere and Peeters, 1997; Furness *et al*., 1999; Nissan *et al*., 2002), but in these studies the concentrations of drug used (100 μM or greater) are considerably higher than those used in the present study or in those by others who describe excitatory functions of erythromycin within the gut.

The temporal nature of responses evoked by the different motilin receptor agonists was studied by measuring the time taken for the prokinetic-like activity to fade back to baseline during their continuous presence. These experiments were time-matched to control tissues in which responses to EFS were measured in the absence of any receptor agonist. Of particular note was the observation that the excitatory responses to both r.motilin and the more stable analogue, [Nle^{13}]motilin, faded rapidly back to baseline during the continuous presence of these peptides. By contrast, the responses to erythromycin faded much more slowly and at the 3 μM concentration, did not return back to baseline during the 60 min period of observation. Interestingly, it was possible to evoke a response on re-application of all but the highest concentration (300 nM) of [Nle^{13}]motilin, following the fade of the original response. The precise magnitude of the second response to [Nle^{13}]motilin is complicated by a time-dependent fall in the baseline amplitude of the EFS-evoked contractions but nevertheless, the experiments demonstrate that a robust response to all but the highest concentration of [Nle^{13}]motilin could still be observed following a prolonged exposure to the peptide. Together, these experiments with the motilin peptides and erythromycin suggest for the first time, an important difference in the way motilin and erythromycin excite enteric nerve activity. The reason for the difference is not understood. One possibility is that there is a difference in the degree of degradation of the peptides relative to erythromycin, although the use of [Nle^{13}]motilin, a more stable analogue of motilin (see earlier discussion), was designed to exclude this possibility, at least partly. Additionally, there were no marked changes in the responses evoked by [Nle^{13}]motilin, measured in the presence of a cocktail of peptidase

inhibitors. Although these experiments still cannot rule out the possibility that both motilin and [Nle^{13}]motilin were rapidly degraded within the tissues via enzymes not affected by the inhibitors used, they do argue for a consideration of alternative possibilities to explain the marked differences observed. It is possible, for example, that the different kinetic responses to motilin and erythromycin are related to the different ways in which these molecules interact with the receptor. Thus, although motilin and erythromycin appear to share a common binding site on the third transmembrane region of the motilin receptor (Xu *et al*., 2005), additional binding sites on the second transmembrane region may be critical for motilin, but not for erythromycin binding to the receptor (Matsuura *et al*., 2002). Accordingly, the rapid loss of an initial response to motilin may be due to a short-lived desensitization of the receptor and/or the downstream, intracellular effector mechanisms, via a specific, peptide-dependent interaction with the motilin receptor.

A rapid loss of response to motilin might be consistent with the relatively aggressive prokinetic-like activity induced by this peptide and also with a proposed role of motilin in the migrating motor complex (Tack, 1995) which by definition, needs to be rapidly self-limiting. By contrast, although it has been suggested that the prokinetic effects of erythromycin *in vivo* may decline with repeated dosing, the literature to support this idea is not clear and indeed, the duration of the response to erythromycin may depend on the dose used. Studies that suggest a possible reduction in the therapeutic benefit of erythromycin after long-term dosing used doses of 250–400 mg, four times a day (Richards *et al*., 1993). However, Dhir and Richter (2004) investigated the effects of a relatively low dose of erythromycin (50–100 mg, three times a day and at bedtime) on symptoms of dyspepsia in patients with gastroparesis, and found a significant correlation between short- and long-term responses to the beneficial effects of this drug. Similarly, symptoms associated with gastroparesis may be improved by repeated intravenous administration of erythromycin, provided the dose was titrated to achieve both efficacy and tolerance in each patient (DiBaise and Quigley, 1999). Finally in a single case report, long-term, low-dose erythromycin (250 mg twice daily for 12 months) was found to be an effective treatment of the vomiting associated with gastric stasis and resistant to cisapride, domperidone and metoclopramide (Hunter *et al*., 2005). These long-lasting prokinetic effects of erythromycin may be reflected by the current experiments *in vitro*, in which the ability of erythromycin to potentiate EFS-evoked contractions faded relatively slowly, compared with motilin. Interestingly, the long-lasting nature of this response to erythromycin contrasts with a short-lasting ability to directly evoke muscle contraction (Dass *et al*., 2003), an assay commonly cited within the literature to support a belief that the prokinetic activity of motilin receptor agonists must be short-lasting (e.g., Thielemans *et al*., 2005). The reasons for this difference are not understood. The use of low concentrations of motilin and erythromycin to activate motilin receptors naturally expressed by neurones within the gut may minimise desensitization of the receptor. Alternatively, if it can be assumed that at all concentrations of all motilin receptor agonists, the receptor is desensitized and possibly internalized (e.g., Lamian *et al*., 2006), then the long-lasting responses observed may be related to maintain changes evoked within the nerves, downstream from the receptor. Further work is required to resolve this difficult question.

In summary, our studies have shown that it is possible to measure and directly compare the prokinetic and desensitization abilities of different motilin receptor agonists and the 5-HT$_4$ receptor agonist, tegaserod, using an assay which reflects the abilities of these agents to increase neuronal activity rather than to contract the muscle directly. This novel approach highlighted marked differences in the maximal activities of tegaserod and the motilin receptor agonists and for the first time, marked differences in the durations of responses to peptide and non-peptide motilin receptor agonists. These data indicate a need for great caution, when using a single agent, to comment on prokinetic drugs in general and especially when judging the potential of motilin receptor agonists as therapeutic drugs.

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Conflict of interest

The authors state no conflict of interest.

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